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Cannabis tea revisited: A systematic evaluation of the cannabinoid composition of cannabis tea

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Abstract

Cannabis is one of the oldest known medicinal plants, and a large variety of biological activities have been described. The main constituents, the cannabinoids, are thought to be most important for these activities. Although smoking of cannabis is by far the most common way of consumption, a significant part of medicinal users consume it in the form of a tea. However, not much is known about the composition of cannabis tea, or the effect of different parameters during preparation, handling or storage. In this study we used the high-grade cannabis available in Dutch pharmacies to study the cannabinoid composition of tea under standardized and quantitative conditions. Experimental conditions were systematically varied in order to mimic the possible variations made by medicinal users. During analysis there was a specific focus on the cannabinoid tetrahydrocannabinol and its acidic precursor, tetrahydrocannabinolic acid. Also the role of non-psychoactive cannabinoids as components of cannabis tea are discussed. The results obtained in this study provide a clear quantitative insight in the phytochemistry of cannabis tea preparation and can contribute to a better appreciation of this mode of cannabis administration.

Keywords: Cannabis; Cannabinoids; Decoction; Medicinal use; Quantitative analysis

1. Introduction

The cannabis plant (*Cannabis sativa* L.) has a long history as herbal medicine, and contains a large variety of pharmacologically interesting constituents. Most important among these are the cannabinoids (Turner et al., 1980), which are unique to the cannabis plant. They are produced by the metabolism of the plant in the form of carboxylic acids (Shoyama et al., 1975), which can be converted into their decarboxylated (neutral) analogs under the influence of light, heat or prolonged storage, by losing the relatively unstable carboxyl-group in the form of CO_2 (Veress et al., 1990). Cannabis can be consumed in a variety of ways, such as smoking, vaporizing, preparing cannabis tea and using it in baked products. A common factor of all administration forms is a heating step, which is essential for conversion of the acidic cannabinoids into the pharmacologically more active neutral ones. The most important conversion that takes place is that of tetrahydrocannabinolic acid (THCA) into delta-9-tetrahydrocannabinol (THC), which is the main bioactive component of cannabis (see Fig. 1).

One popular way to undergo the effects of cannabis is by consuming it in the form of a decoction, which will be referred to in this manuscript as 'cannabis tea'. In Jamaica, which is sometimes quoted as the country with the highest consumption of cannabis, the different uses of cannabis have been thoroughly studied (Rubin and Comitas, 1975). Although cannabis, which is locally known as ganja, is mostly consumed by smoking, drinking of ganja tea is common among non-smokers (Boekhout van Solinge, 1996) and is consumed even by young children and the elderly. The tea is attributed various therapeutic and prophylactic qualities and is used as a remedy for fever, cold and stress.

Also around Europe, hemp-containing foods, including leaves for tea preparation, are widely available. Often these products are associated with health. Although it is legally not permitted, herbal hemp leaves used for tea have been found to contain high THC levels (1020–5000 mg/kg) and significant concentrations were determined in the corresponding tea infu-

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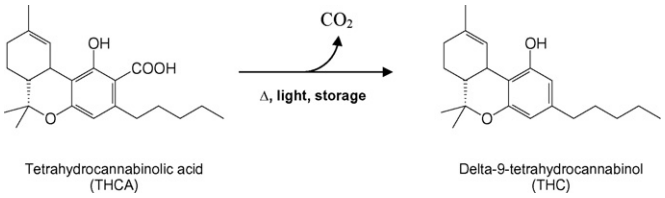

Fig. 1. Conversion of THCA into THC, as it is taking place during the preparation of tea. The same conversion also happens, more slowly, as the result of storage and aging.

sions (1.0–2.4 mg/L) (Giroud et al., 1997; Zoller et al., 2000). Potentially, any health claims based on the consumption of such teas might therefore be attributable to its content of THC. After all, positive drug tests for cannabis use as well as intoxication have been reported after ingestion of such products (Struempler et al., 1997), and analytical methods have been developed for the forensic screening of THC in these products (Lachenmeier et al., 2004).

In contrast, other (non-psychoactive) cannabinoids usually go undetected and might be present in any concentration in officially allowed hemp products, including tea. For example, the major cannabinoids cannabidiol (CBD) and cannabinol (CBN) can be found in most cannabis cultivars, and both have reported biological effects, such as antibacterial and anti-inflammatory activity, and modulation of immune responses (Grotenhermen and Russo, 2002). The potent immuno-modulating properties of the major cannabinoid THCA have only recently been discovered (Verhoeckx et al., 2006). These effects clearly make the non-psychotropic cannabinoids potential candidates for any medicinal claims attributed to the consumption of cannabis tea.

However, with few exceptions (Steinagle and Upfal, 1999; De Jong et al., 2005) virtually no standardized studies have been performed with tea preparations of cannabis. The single large scale field study which includes the use of cannabis tea (Rubin and Comitas, 1975) lacks a focus on analytical data, such as chemical composition and potency of cannabis used, making it difficult to understand the effects or reliability of this administration form. Clearly, there is a need for a better understanding of the composition of cannabis tea prepared under varying conditions, before further conclusions can be made on its effects or reliability.

Recently, the introduction of high grade cannabis for medicinal use in The Netherlands has provided a good opportunity to study the composition of cannabis tea. The detailed conditions of this introduction, through the Dutch Office of Medicinal Cannabis (OMC), have been previously described (Hazekamp, 2006). Under the Dutch regime, patients essentially are able to freely choose their manner of cannabis consumption. Based primarily on health implications, the OMC advices to consume medicinal cannabis preferably by vaporizing or in the form of a tea. Indeed, polls under medicinal cannabis users in The Netherlands have indicated tea preparation to be a popular way of consuming cannabis (Janse et al., 2004).

Considering these developments, a systematic study on the composition of cannabis tea would be very interesting. We performed this phytochemical study on the preparation and handling of cannabis tea, in particular on the parameters that can have an effect on the composition of the tea, such as boiling time, volume of tea prepared, and duration of storage. In order to understand the magnitude of such effects, parameters were systematically varied to determine their effect on the cannabinoids present in the tea, with a particular focus on the main cannabinoids THC and THCA. To improve the observed poor stability of tea during refrigerated storage, we evaluated the use of solubilizers. Finally, we discuss the potential role that the non-psychoactive cannabinoids may play in the effects attributed to cannabis tea.

2. Materials and methods

2.1. Materials

Cannabis plant material used in this study was of the variety ‘Bedrocan’ and was obtained from Bedrocan BV (Veendam, The Netherlands) where it was cultivated under standardized conditions according to the requirements of Good Agricultural Practice (GAP) (Hazekamp, 2006). Only female flower tops were used (‘Cannabis Flos’). After harvest, the plant material was air-dried in the dark under constant temperature and humidity for 1 week. The same cannabis material is officially dispensed through Dutch pharmacies under the Dutch medicinal cannabis program, supervised by the Office of Medicinal Cannabis (OMC). This cultivar is of the drug-type (Fetterman et al., 1971) and at the time of use it had a THCA content of 191 mg/g (19.1%), and a THC content of 6 mg/g (0.6%) of dry weight plant material.

Pure ethanolic standards for THC and THCA were produced as previously described (Hazekamp et al., 2004a,b). Randomly-methylated (RM)-beta-cyclodextrin was obtained from Wacker Chemie GmbH (Burghausen, Germany) and was used as received.

All organic solvents were HPLC or analytical grade and were purchased from Biosolve (Valkenswaard, The Netherlands). Water used for tea preparation was regular tap-water.

2.2. Preparation of tea samples

The users of medicinal cannabis in The Netherlands are advised by the OMC to prepare cannabis tea according to the following standard protocol: “add 1.0 g of cannabis to 1.0 L of boiling water and let simmer for 15 min. Filter out solid parts by using a common tea-sieve. Tea can be consumed immediately, or stored in a closed bottle in a refrigerator for up to 5 days” (OMC, 2006). Throughout this study, tea prepared according to this protocol is referred to as ‘standard tea’, and it is the reference material for all performed tests.

Tea was prepared in 2 L glass Erlenmeyer flasks on an electronic heating plate. For each experiment, three separate preparations were made, unless stated otherwise. Samples for analysis were taken after the tea was allowed to cool down to a temperature of about 55 °C. After shortly stirring up the tea, from each preparation a sample of 30 mL was collected by pouring the

liquid through a common metal tea-sieve into a calibrated measuring cylinder. Samples were lyophilized to complete dryness and reconstituted in ethanol for analysis.

2.3. Determination of cannabinoids

Cannabinoid content of the tea samples was determined by high-pressure liquid chromatography (HPLC), as described before (Hazekamp et al., 2004a). The HPLC method was validated according to recent ICH guidelines (ICH, 2006) for the quantitative analysis of cannabinoids in extracts of herbal cannabis. Pure ethanolic standards of the cannabinoids were used for quantitation.

2.4. Stability and recovery of THCA and THC standards

Stability and recovery of the main cannabinoids THC and THCA during preparation of samples for analysis was studied by standard addition (spiking) of pure cannabinoids to boiling water, in concentrations that were similar to those found in standard tea. Water with added standards was processed as described for regular tea samples. For THCA, its conversion rate into THC was determined.

2.5. Variability of standard tea

The variability in the composition of standard tea was determined by analyzing six different preparations of standard tea (1 L) and calculation of relative standard deviation (%S.D.) of cannabinoid levels. Because the levels of THC and THCA are commonly considered most important for bioactivity, these cannabinoids were analyzed quantitatively. Other cannabinoids were analyzed only qualitatively, based on HPLC peak area, so without the use of calibrated standards.

The herbal cannabis material that remained after tea preparation (residue after filtering by the sieve) was extracted with ethanol in order to determine its cannabinoid composition by HPLC analysis. Obtained data was used to determine the mass balance for the distribution of THC and THCA before and after preparation of standard tea.

2.6. Effect of preparation parameters on tea composition

Changing the preparation parameters may have an effect on the composition of the tea, both on the absolute concentration and on the relative ratio of cannabinoids that are found in the tea. We tested the effect of systematically changing each of the parameters described below. Effects were statistically evaluated by using the independent Student's *t*-test with two-tailed distribution:

- *Volume*. Tea was prepared with 250 mg of cannabis in 250 mL of water versus 1.0 g in 1.0 L of water. The 250 mL preparations were made in 500 mL glass Erlenmeyer flasks.
- *Amount of cannabis*. Tea was prepared using 0.5, 1.0 and 1.5 g of cannabis.
- *Boiling time*. Tea was prepared by boiling for 10, 20 and 30 min. The influence of evaporation of water during boiling was not evaluated in this study, but this factor was kept to a minimum by loosely covering the opening of the flask.

2.7. Storage and stability

Based on microbial spoiling, it is claimed that medicinal cannabis tea can be stored in a refrigerator for a maximum of 5 days (OMC, 2006). To test the effect of storage on the THC and THCA concentration of standard tea, multiple samples of 50 mL were taken from a single preparation of tea, and stored in a refrigerator (+4 to +7 °C) for periods of 1, 3, 5 and 12 days. After this period, samples were gently stirred, and 30 mL was removed for analysis.

Samples that had been stored for 3 days were used for analysis of the precipitate that had formed. Samples were gently stirred and subsequently the water phase was poured off. Residue that remained in the storage tube was dissolved in ethanol for quantitative analysis of THC and THCA. Obtained data was used to determine the mass balance for the distribution of THC and THCA before and after storage of standard tea.

2.8. Effect of solubilizers

An important drawback of tea preparation is the very limited solubility of cannabinoids in water (Garrett and Hunt, 1974; Hazekamp and Verpoorte, 2006). In order to stabilize the composition of cannabis tea, the addition of solubilizers was evaluated. Previous studies have shown that the addition of cyclodextrins is a promising way to increase the water solubility of several cannabinoids, including THC and THCA (Mannila et al., 2005; Hazekamp and Verpoorte, 2006), suggesting that addition of cyclodextrins can stabilize the levels of THC and THCA during storage. Therefore, the addition of 1% and 3% (w/v) of randomly methylated beta-cyclodextrin (RAMEB) to standard tea was evaluated. Other common types of cyclodextrins were previously shown to be ineffective in improving the aqueous solubility of THC (Hazekamp and Verpoorte, 2006). Addition was done directly after preparation and tea (200 mL) was stored in a refrigerator for 5 days.

Another solubilizer tested was coffee creamer powder, which was added to cannabis tea (one standard package per cup; ±2.5 g per 200 mL) while still warm. Tea was stirred until powder was completely dissolved, before refrigerated storage for 5 days.

3. Results and discussion

3.1. Behaviour of pure cannabinoids in boiling water

In order to understand the composition of cannabis tea, initially some studies were done with pure cannabinoid standards. Recovery of THC and THCA during sample preparation for HPLC analysis (i.e.: lyophilization and reconstitution of tea samples) was found to be 79.8% (±4.5%) for THC and 94.8% (±0.5%) for THCA. All subsequent measurements were corrected for these values.

When pure THC was added to boiling water, only about 17% was recovered after 15 min of boiling. A THC precipitate was clearly visible on the surface of the glass flask used for boiling the water, indicating that a saturated solution had formed. Spiking of pure THCA resulted in a much higher recovery of about 63%. A small part of added THCA could be recovered from the water phase in the form of THC (6.6%), the remaining part was found as a precipitate on the glass container used for boiling.

These results indicate that conversion of THCA into THC is limited in boiling water. Furthermore it is suggested that a saturated THC solution forms in boiling water, implicating that addition of extra THC will probably not increase its water concentration. Similar observations were made when analyzing cannabis tea samples (see below).

Boiling of the standards did not results in the formation of degradation products such as CBN or delta-8-THC, indicating that degradation of these major bioactive cannabinoids is not a significant factor during tea preparation.

3.2. Composition of standard tea

Analysis of six different batches of standard tea showed that the variability in the composition of standard tea is relatively low for a preparation method that is essentially very crude: variability for the content of THC (mean: 0.010 mg/mL) was 15% while for THCA (mean: 0.043 mg/mL) it was only 12%. Other cannabinoids visible in the HPLC chromatogram were analyzed only qualitatively (based on relative HPLC peak area). A typical HPLC chromatogram obtained during analysis of standard tea is shown in Fig. 2. Variability was found to be in the range of 8.4–17.4% for all cannabinoids.

3.3. Mass balance of THC and THCA

By calculation of total THC (sum of THC and THCA, taking into consideration the difference in molecular weight) present, the mass balance of THC before and after tea preparation was determined. It was found that no net loss of THC occurred during tea preparation: total THC present in the plant material before preparation (174 mg) was found to be equal to the amount present (in water phase plus in residual plant material) directly after preparation (176 mg). These results indicate that loss of THC by degradation does not play a significant role. Indeed, no degradation products of THC were observed during the experiments with pure cannabinoids, as described above.

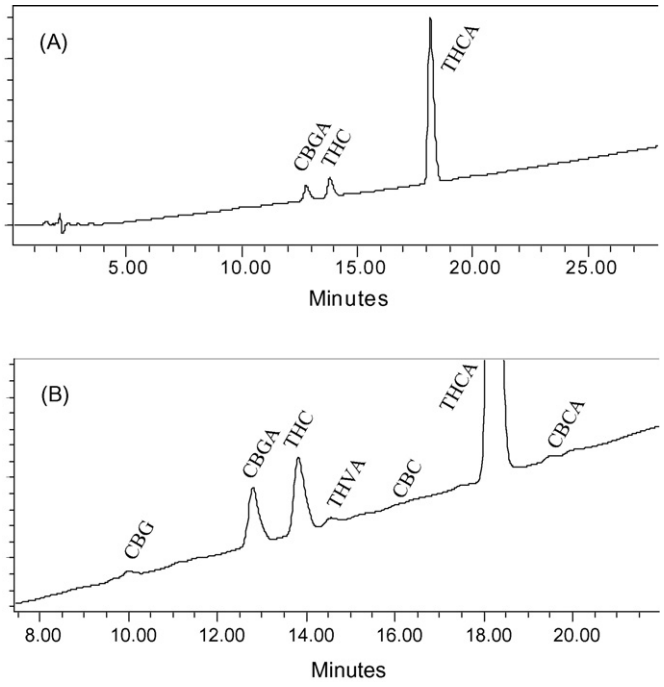

Fig. 2. Typical HPLC chromatogram (228 nm) obtained by analysis of standard cannabis tea according to the method described: (A) whole chromatogram and (B) enlargement of the cannabinoid peaks.

3.4. Effect of preparation parameters

Studying the effects of changing the basic parameters of tea preparations gave a good insight into the behaviour of both THCA and THC during the preparation process. Results are summarized in Fig. 3. Differences with a significance of $p < 0.05$ are indicated.

- *Volume*. No significant differences were found between tea prepared in a volume of 1 L or 250 mL; THC and THCA

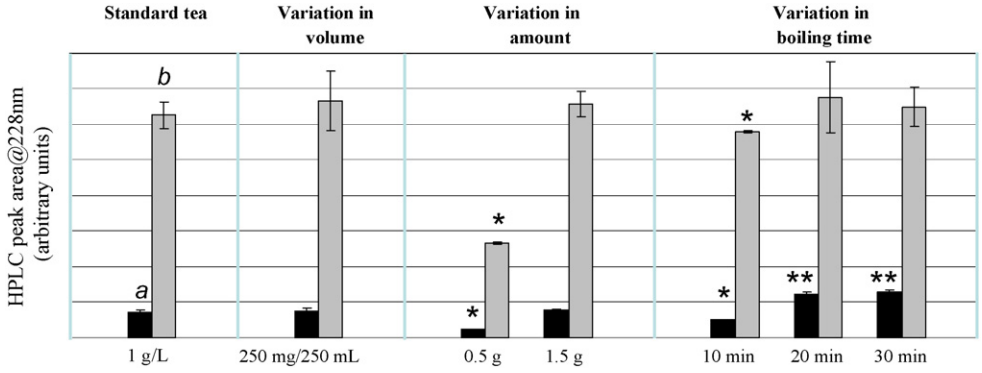

Fig. 3. Effect of variations in water volume, amount of cannabis, and boiling time used in the preparation of cannabis tea. The levels of THC and THCA are expressed in units of peak area (HPLC at 228 nm). a: Bar corresponds to a THC level of 0.010 mg/mL; b: bar corresponds to a THCA level of 0.043 mg/mL; *: significantly lower than standard tea; **: significantly higher than standard tea; $p < 0.05$.

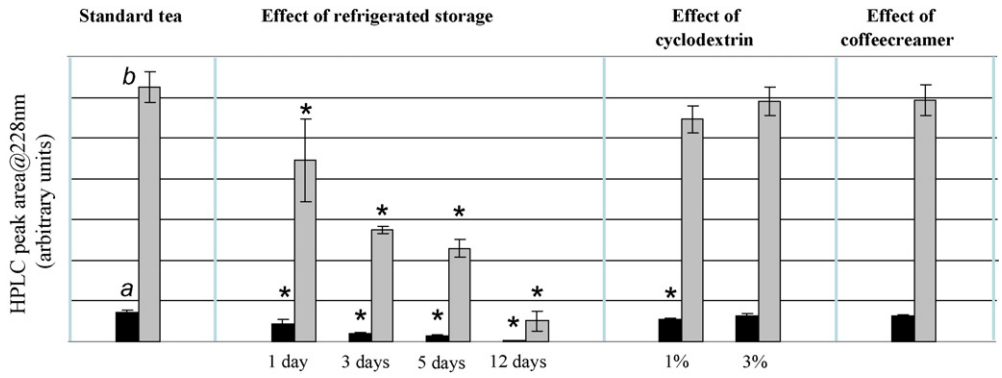

Fig. 4. Effect of prolonged refrigerated storage of standard cannabis tea, without and with addition of solubilizers. The levels of THC and THCA are expressed in units of peak area (HPLC at 228 nm). a: Bar corresponds to a THC level of 0.010 mg/mL; b: bar corresponds to a THCA level of 0.043 mg/mL; *: significantly lower than standard tea; $p < 0.05$.

levels, as well as general profile of cannabinoids were similar directly after preparation of the tea, and also after 5 days of refrigerated storage (data not shown). These results indicate that downscaling of the volume of tea does not influence the composition of the final product.

- *Amount of cannabis*. The use of a higher than usual amount of cannabis (1.5 g) did not significantly increase the aqueous concentration of THC or THCA, compared to the use of 1.0 g, again suggesting that a saturated solution forms. In contrast, the use of half the usual amount (0.5 g) of cannabis significantly decreased the water concentration of both THC and THCA to about half the concentration found for standard tea.
- *Boiling time*. Variation in boiling time in the range of 10–30 min had only a slight effect on the level of THCA; levels found were similar at all tested boiling times. In contrast, the level of THC was found to be dependant on boiling time, as increased boiling time resulted in significantly higher levels of THC. However, THC levels remained much lower than THCA levels found in these preparations.

3.5. Effect of storage and solubilizers

Refrigerated storage resulted in steady decrease of cannabinoid levels (Fig. 4). Even after a single day of storage, concentrations of THC and THCA had significantly decreased to 60% and 71% of initial levels, respectively. After 12 days of storage, these values had decreased further to only 6% and 8% of initial values, respectively. After preparation, when the tea cools off, the liquid is observed to turn from clear to opaque, indicating formation of a precipitate. Analysis of this precipitated matter after 3 days of storage showed that the amount of THC and THCA recovered from the precipitate was equivalent to the amount lost from solution. However, the relative cannabinoid composition did not change very much during the same period, meaning that all cannabinoids present precipitated roughly to the same extent. In other words, the potency decreased while the qualitative composition remained the same.

It was found that addition of cyclodextrin as well as coffee creamer was effective in stabilizing cannabis tea during refrigerated storage (see Fig. 4). After 5 days the levels of THC and THCA in the tea were found to be virtually unchanged. Addition of 3% of RAMEB had a slightly better stabilizing effect than addition of 1% of this compound.

4. Conclusion

Cannabis tea can be considered as a contemporary example of a widely used, but poorly understood herbal medicine. A major concern with the medicinal use of cannabis is the risk of (accidental) overdosing of THC, which could lead to psychotropic effects. However, our results show that moderate changes in the standard preparation protocol for cannabis tea do not result in dramatic changes in the composition of the tea, neither quantitatively nor qualitatively. Rather, the results indicate that cannabis tea has only limited potency, and that probably a saturated solution of THC forms.

By performing a series of experiments, we systematically discovered the effect of different parameters on the cannabinoid composition of medicinal cannabis tea. The study of pure standards in boiling water provided detailed insight into the behaviour of THC and THCA during the tea preparation process. Relatively, more THCA was solubilized in boiling water than THC, which probably can be understood by the relatively higher water solubility of THCA compared to THC (Hazekamp and Verpoorte, 2006). Interestingly, although the amount of THC in the used amount of cannabis (1 g) is potentially very high (about 174 mg, as sum of THC and THCA), the whole volume of standard tea contains only a fraction of this (about 10 mg THC per liter) in the water phase. This relatively low concentration is probably the result of saturation of the water phase with THC, in combination with a moderate conversion of THCA into THC, as was also suggested by the experiments performed with pure standards.

In case storage of cannabis tea is required, the addition of a solubilizer was found stabilize the THC and THCA levels of the preparation for a period of at least 5 days. Although addition of the cyclodextrin RAMEB clearly improved the stability of cannabis tea, its oral use has not yet been fully validated and its common use in medicinal preparations might still take several years to be established. However, the addition of coffee creamer can be an easy and safe alternative for medicinal consumers

of cannabis tea to stabilize their preparation during short-term storage.

Finally, some attention should be given to the unique composition of cannabis tea, compared to other forms of administration, where heating of the material is typically performed at much higher temperatures (e.g. smoking, vaporizing or baking), resulting in a virtual complete conversion of acidic into neutral cannabinoids. This is the reason that, during studies into the medicinal effects of cannabis preparations, the attention is commonly focussed on THC alone. However, in the cannabis tea studied, a significant proportion of THCA was found. The recently described immuno-modulating properties of THCA (Verhoeckx et al., 2006) may contribute to the effects that certain groups of medical users claim after consumption of cannabis tea. Furthermore, a variety of other acidic cannabinoids were found by HPLC analysis, such as cannabigerolic acid (CBGA) and tetrahydrocannabivarinic acid (THVA). Although the biological activities of these compounds have hardly been explored, their presence makes cannabis tea a unique administration form that should not be considered as simply a vehicle for THC.

In conclusion, cannabis tea is already consumed by a large number of patients on a daily base, and their medical claims may certainly be compatible with the unique composition of the tea. The results obtained in this study can contribute to a better understanding of cannabis tea, resulting in a better appreciation of this popular form of cannabis administration.

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